Effects of Replacement of Native Fat in Colostrum and Milk with Coconut Oil on Fat-Soluble Vitamins in Serum and Immune Function in Calves

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ABSTRACT

Fat-soluble vitamins and their metabolites modulate immune function in a variety of animal species. The objective of the present study was to determine the role of fat-soluble vitamins in colostrum and milk in the development of specific aspects of immune function in the calf during the 1st wk postpartum. During this period, control calves (n = 6) were fed normal colostrum and milk, and calves in the treatment group (n = 6) were fed skimmed colostrum and skimmed milk supplemented with coconut oil. Treated calves did not experience the progressive increase in concentrations of retinol, \( \beta \)-carotene, \( \alpha \)-tocopherol, 1,25-dihydroxyvitamin D, or retinoic acids in serum that was observed in control calves. Acquisition of passive immunity, which is indicated by concentrations of immunoglobulin G1 in serum, was unaffected by treatment. Composition and functional capacities of populations of blood mononuclear leukocytes that were collected from birth to 7 d postpartum were also unaffected by treatment. Major changes in the function and composition of mononuclear leukocyte populations from all calves occurred during the experimental period and were unrelated to the concentrations of fat-soluble vitamins in serum. Populations of blood mononuclear leukocytes from calves were functionally hyporesponsive and compositionally different from populations of blood mononuclear leukocytes from adult nongravid cows. These differences likely reflected the immaturity of the immune system of the neonatal calf and may contribute to the increased susceptibility of the calf to infectious disease.

(\textit{Key words}: immune function, calf, vitamin A, nutrition)

INTRODUCTION

Physiologic immaturity of the neonatal immune system is thought to render the newborn more susceptible to infectious diseases than the adult (5). Studies with humans and mice indicate that this immaturity is characterized by the presence of populations that suppress Ig production higher proportions of naive T cells and T-cell (10), higher proportions of antigen-presenting cells with defective costimulatory activity (35), and decreased abilities to produce cytokines (36). Calves are known to have higher proportions of \( \gamma \delta \) T cells (14) and lower proportions of circulating B cells (37) than do adult cows. By about 20 wk of age, the number of B cells (38) and the ability of the calf to produce Ig (24) gradually increase to levels observed in adults. Serum from 1-d-old calves contains factors other than cortisol that suppress lymphoblastogenesis of calf lymphocytes from the spleen, thymus, and lymph nodes (22).

Newborn calves have very low concentrations of vitamin A metabolites and \( \alpha \)-tocopherol in serum. As age increases, these components increase, and adult concentrations are attained by about 2 yr of age (15). Colostrum, milk, and formulated diets are major sources of these vitamins for the neonatal calf (38). Vitamin A is known to promote differentiation and maturation of a variety of cell types, primarily through its metabolites, the retinoic acids (RA), which are derived from retinol and retinyl esters (23). \( \beta \)-Carotene is not only an antioxidant, but also a source of vitamin A (8). Vitamin E is recognized for its role as an antioxidant in conjunction with Se (15), and vitamin D acts via its metabolite, 1,25-dihydroxyvitamin D \( [1,25(\text{OH})_2\text{D}] \), to maintain Ca homeostasis in the calf (16). These vitamins and

1. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.
2. To whom reprint requests should be addressed.
their metabolites have also been shown to modulate immune parameters in cattle and other species. However, little information is available regarding the status of fat-soluble vitamins and their impact on the immune responsiveness of the newborn calf. Retinoic acids and 1,25(OH)2D promote the differentiation and maturation of cells of myeloid origin (4). Studies with mice and humans (7, 20) indicate that these compounds preferentially inhibit functions of Th1 cells, which promote cell-mediated immune responses. Metabolites of retinol are essential for B-cell growth and activation (3). In adult cattle, in vitro supplementation with RA isomers or 1,25(OH)2D enhance IgM production by blood mononuclear leukocytes (MNL) (30, 32). Both 13-cis-retinoic acid and 1,25(OH)2D inhibit lymphocyte proliferation and expression of activation markers by mitogen-activated MNL from adult dairy cattle (26, 28). 1,25-Dihydroxyvitamin D inhibits interferon-γ (IFN-γ) production by peripheral blood mononuclear cells from cows (1). In cattle, vitamin E supplementation has been associated with increased specific antibody titers after vaccination and enhanced in vitro T- and B-cell mitogenesis, IgM, and interleukin-1 production (9). β-Carotene enhances blastogenic responses of lymphocytes and increases cytotoxic activities of natural killer cells and cytokine production by macrophages (8).

Recently, 9,13-di-cis-retinoic acid has been identified as the most abundant vitamin A metabolite in the plasma of the neonatal calf and in the cow around parturition (17, 18). Elevation of plasma 9,13-di-cis-RA in the neonatal calf is dependent on the consumption of whole colostrum. Although 9,13-di-cis-RA has little biologic activity (17, 31, 33), studies with rats (18, 39) indicate that 9-cis-RA, which is metabolically active, may be a precursor to 9,13-di-cis-RA. These observations suggest that events in vitamin A metabolism occur in the neonatal calf or dam around the time of parturition, leading to the generation of retinoids that have the potential to influence immune responsiveness in the newborn calf or dam. The objective of this study was to develop an in vivo model in which the elevation in fat-soluble vitamins could be prevented to enable us to study the effects of those vitamins on immune parameters in the neonatal calf. To achieve this objective, newborn calves were fed colostrum and milk in which the native fat, which contains fat-soluble vitamins or their precursors, was replaced with coconut oil, a fat source that lacks detectable fat-soluble vitamins. Several immune parameters were monitored during the 7-d experimental period.

MATERIALS AND METHODS

Calves and Diet

Twelve newborn Jersey calves of cows housed at the USDA, ARS, National Animal Disease Center were caught at birth before suckling the dam. During the 1st wk postpartum, 6 calves were fed complete colostrum and milk, and 6 calves were fed skimmed colostrum and milk that were supplemented with coconut oil [12 and 3.5%(vol/vol), respectively; volumes were similar to the amount of native fat in whole colostrum and milk]. Colostrum was collected from 10 cows and pooled. Approximately one-half of the volume of pooled colostrum was skimmed by centrifugation. Intact and skimmed colostrum was divided into 4-L aliquots and frozen at -20°C. Each aliquot was thawed immediately before use. Each calf was fed 2 L of pooled colostrum within 3 h of birth and another 2 L within 12 h of birth. Calves were subsequently fed 2 L of milk in the morning and afternoon each day. Concentrations of fat-soluble vitamins in the diets were measured by HPLC (17, 18).

Calves remained clinically normal throughout the experimental period. Procedures involving the calves were approved by the Institutional Animal Care and Use Committee of the USDA, ARS, National Animal Disease Center.

Blood Collection and MNL Isolation

Blood samples were obtained by jugular venipuncture of calves within 3 h after birth and once every 24 h for 7 d thereafter. When a calf was born after 1200 h, blood that had been collected was held at room temperature (20°C) in the dark and processed the next morning. Blood collected before 1200 h was processed the same day. Blood from 2 adult, non-gravid, nonlactating Jersey heifers was collected on eight separate occasions during the study and was processed the same way. Blood was collected into 10% (vol/vol) 2× solution of acid, citrate, and dextrose. Blood MNL were isolated and enriched by density gradient centrifugation as described previously (27). Contaminating erythrocytes were eliminated by hypotonic lysis prior to density gradient centrifugation of buffy coat cells. Enriched MNL were resuspended in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) that was supplemented with 2 mM L-glutamine (Sigma Chemical Co., St. Louis, MO), antibiotics (100 U/ml of penicillin G sodium and 100 μg/ml of streptomycin sulfate), and antimycotics (0.25 μg/ml of amphotericin B; Gibco Laboratories). The

medium used for IFN-γ assays was also supplemented with nonessential amino acids (catalog no. M-7145; Sigma Chemical Co.) and 2-mercaptoethanol (55 μM; Gibco Laboratories).

**Measurement of Metabolites and Immunoglobulin G₁ in Serum**

Serum samples were collected to quantify concentrations of retinol, β-carotene, α-tocopherol, and RA by HPLC as described by Horst et al. (17, 18). Concentrations of 25-hydroxyvitamin D \([25(OH)D]\) in serum were determined by radioimmunoassay using a kit (reference no. 68100; IncStar Corp., Stillwater, MN). A nonequilibrium receptor-based assay was used to determine concentrations of 1,25(OH)₂D in serum as was described by Reinhardt et al. (34). Concentrations of IgG₁ in serum were determined by an ELISA that was similar to the procedure used to determine IgM (29).

**Phenotype Analysis of MNL Populations**

Leukocytes in MNL populations were phenotyped by modifications of the flow cytometry procedure described previously (26). Density gradient-enriched MNL were washed with and resuspended in cold Hanks balanced salt solution (HBSS) with 1% heat-inactivated fetal calf serum (FCS; Hydene Laboratories, Inc., Logan, UT) and 0.1% NaN₃ at a density of 10 × 10⁶/ml. Approximately 5 × 10⁵ MNL from each suspension were added to individual wells of a 96-well microtiter plate. Monoclonal antibodies (VMRD, Inc., Pullman, WA) (Table 2), diluted in HBSS with 1% FCS and 0.1% NaN₃, were added (50-μl aliquots) individually to wells containing the MNL. Plates were incubated at 4°C for 15 min and were washed twice by centrifugation (1171 × g at 4°C for 2 min). Supernatants were removed using a plate washer (Dynatech Miniwash; Dynatech Laboratories, Alexandria, VA). Cells were resuspended in HBSS with 0.1% NaN₃ and incubated (4°C for 15 min) with secondary antibody [fluorescein isothiocyanate-conjugated goat F(ab')₂ fragments against mouse IgG or IgM, 50 μl; Organon-Teknika-Cappel, Durham, NC]. Plates were washed again, and cell pellets were resuspended in HBSS with 0.1% NaN₃. Nonspecific binding of antibody was assessed by incubating each test sample with secondary antibody alone. Specificities of all primary antibodies were ensured by testing with isotype controls.

A FACScan® (Becton Dickinson Immunocytometry Systems, San Jose, CA) was used for flow cytometric analysis of 5000 cells that exhibited light scattering properties that were consistent with bovine MNL. An argon laser with an excitation wavelength of 488 nm was used to detect cells associated with fluorescein isothiocyanate-conjugated second antibody. Emission fluorescence was detected with a 530-nm bandpass filter and converted to log fluorescence. Markers were positioned for negative control samples to provide a background of ~2% and were maintained at this position for all samples taken from the calf. Cells with fluorescence intensity greater than the marker position were considered positive. The fluorescence data associated with each parameter were expressed as a percentage of the gated MNL population.

**In Vitro Production and Measurement of IgM**

Secretion of IgM by unstimulated MNL cultures and MNL cultures stimulated with pokeweed mitogen (PWM) was quantified by an ELISA as described previously (29). Cultures were established in flat-bottomed, 24-well polystyrene tissue culture plates inoculated with 1.0 × 10⁶ cells/ml in a final volume of 1.5 ml containing 5% (vol/vol) FCS in RPMI 1640 medium with antibiotics, antimycotics, and glutamine. Resting MNL cultures and MNL cultures stimulated with PWM (0.08 and 0.32 μg/ml, respectively) were incubated at 39°C in a humidified atmosphere containing 5% CO₂ for 14 d. The concentration (nanograms per milliliter) of IgM in supernatants was determined by comparison of absorbance of supernatants with absorbance of standards within a linear curve fit.

**In Vitro Production and Measurement of IFN-γ**

Secretion of IFN-γ was evaluated in MNL cultures established in flat-bottomed, 96-well polystyrene tissue culture plates inoculated with 5 × 10⁶ cells/ml in a total volume of 200 μl of RPMI 1640 medium with 5% FCS, 55 μM 2-mercaptoethanol, and nonessential amino acids. Resting MNL cultures and MNL cultures stimulated with PWM (10 μg/ml) were incubated for 48 h at 39°C in a humidified atmosphere with 5% CO₂. Culture supernatants from centrifuged plates (800 × g at 4°C for 2 min) were harvested and stored at −80°C until analyzed. Interferon-γ was measured using an IFN-γ capture ELISA as reported previously (1). Absorbance of standards, controls, and test samples was determined at 410 and 490 nm (test filter, 410 nm; reference filter, 490 nm) using an automated ELISA plate
Concentrations of fat-soluble vitamins in the diets

Concentrations (nanograms per milliliter) of fat-soluble vitamins in the diets fed to the calves are indicated in Table 1. Normal milk had 3 to 4-fold higher retinol than did colostrum. Skimmed milk had very low concentrations of retinol. Normal colostrum and milk had about 8 to 13-fold higher \( \alpha \)-tocopherol and 1.4 to 1.6-fold greater concentrations of 1,25-(OH)\(_2\)D than did skimmed colostrum and skimmed milk. Normal colostrum had 4.5-fold higher \( \beta \)-carotene than did skimmed colostrum. No \( \beta \)-carotene was detected in skimmed milk. There was no detectable 25(OH)D in normal or skimmed colostrum or in normal or skimmed milk. These vitamins were not detectable in coconut oil (data not shown).

Statistical Analysis

Data were analyzed by a split-plot repeated measures ANOVA. The statistical model included effects of in vivo treatment, day, and the interaction of day and treatment. The mean square error for the calf (treatment) was used as the error term (i.e., as the denominator for treatment calculated from the Type III sums of squares) to evaluate the effect of treatment. Residual error was used as the error term to evaluate other effects. Effects of in vitro treatments of the MNL were evaluated by a split-plot repeated measures ANOVA in which treatments were blocked by calf. The model included the main effects of mitogen. The error term used to evaluate these effects was the mean square error for the interaction of calf and mitogen. Effects of day and its interaction with mitogen were assessed using the residual as the error term. Significant differences between treatments or days were calculated by Student’s two-sample t test using least squares means. Statistical significance was declared at \( P < 0.05 \). Pearson’s correlation coefficients were calculated between variables. Correlations were declared significant at \( P < 0.05 \). Data for the DNA synthesis assay were log\(_{10}\) transformed prior to analysis.

RESULTS

Concentrations of Fat-Soluble Vitamins in the Diets

Concentrations (nanograms per milliliter) of fat-soluble vitamins in the diets fed to the calves are indicated in Table 1. Normal milk had 3 to 4-fold higher retinol than did colostrum. Skimmed milk had very low concentrations of retinol. Normal colostrum and milk had about 8 to 13-fold higher \( \alpha \)-tocopherol and 1.4 to 1.6-fold greater concentrations of 1,25-(OH)\(_2\)D than did skimmed colostrum and skimmed milk. Normal colostrum had 4.5-fold higher \( \beta \)-carotene than did skimmed colostrum. No \( \beta \)-carotene was detected in skimmed milk. There was no detectable 25(OH)D in normal or skimmed colostrum or in normal or skimmed milk. These vitamins were not detectable in coconut oil (data not shown).

Serum Concentrations of Vitamins, Metabolites, and IgG\(_1\) in Serum

At birth (d 0), concentrations of retinol, \( \beta \)-carotene, \( \alpha \)-tocopherol, RA isomer, 25(OH)D, and

<table>
<thead>
<tr>
<th>Source</th>
<th>Retinol (ng/ml)</th>
<th>( \beta )-Carotene (ng/ml)</th>
<th>( \alpha )-Tocopherol (ng/ml)</th>
<th>25(OH)D(_1) (ng/ml)</th>
<th>1,25(OH)(_2)D(_2) (ng/ml)</th>
</tr>
</thead>
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<tr>
<td>Colostrum Normal</td>
<td>36</td>
<td>317</td>
<td>4602</td>
<td>ND(^3)</td>
<td>0.0677</td>
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<tr>
<td>Skimmed</td>
<td>28</td>
<td>70</td>
<td>365</td>
<td>ND(^3)</td>
<td>0.0496</td>
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<tr>
<td>Milk</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>103</td>
<td>5</td>
<td>1819</td>
<td>ND(^3)</td>
<td>0.0388</td>
</tr>
<tr>
<td>Skimmed</td>
<td>5</td>
<td>ND</td>
<td>216</td>
<td>ND(^3)</td>
<td>0.023</td>
</tr>
</tbody>
</table>

\(^1\)25-Hydroxyvitamin D.
\(^2\)1,25-Dihydroxyvitamin D.
\(^3\)Not detected.
1,25(OH)$_2$D were the same in the serum of control and treated calves (Figures 1, 2, and 3). Thereafter, there were progressive increases in concentrations of these compounds in serum of control calves, but, in calves fed a diet of skimmed colostrum or skimmed milk, concentrations of these compounds remained relatively unchanged over the 7-d period. From d 1 to 7, calves fed skimmed colostrum and skimmed milk had significantly lower concentrations of retinol ($P \leq 0.007$; Figure 1a), $\beta$-carotene ($P \leq 0.04$; Figure 1b), and 9,13-di-cis-RA ($P \leq 0.0005$; Figure 2a) in serum relative to concentrations in the serum of control calves. Treated calves also had lower concentrations of $\alpha$-tocopherol ($P \leq 0.01$; Figure 1c) from d 2 to 7 and lower concentrations of 1,25(OH)$_2$D ($P \leq 0.01$; Figure 3b) in serum from d 3 to 7. Although 9-cis-RA, all-trans-RA, and 13-cis-RA concentrations in serum were $<2$ ng/ml for calves in both groups during the experimental period, treated calves frequently had lower ($P \leq 0.05$) concentrations of these isomers (Figure 2, b, c, and d).

Concentrations of IgG$_1$ in serum were unaffected by treatment ($P > 0.05$; Figure 4). Immunoglobulin
G₁ was undetectable at birth, increased to >1500 mg/dl by d 2, and remained unchanged for the duration of the experimental period in control and treated calves.

**Composition of MNL Populations**

The composition of MNL populations, secretion of polyclonal IgM and IFN-γ, and DNA synthesis by MNL were unaffected by the diet based on coconut oil (P > 0.05). The results described subsequently are representative of calves from both treatment groups.

Percentages of MNL that expressed specific cell-surface markers are shown in Table 2. There were significant (P = 0.0001) day-related changes in the percentage of positive MNL that expressed CD3+, γδ T cell, B cell, and major histocompatibility complex (MHC) class II+ antigens. The other antigens did not change with time. The MNL populations from 1-d-old calves had higher percentages of CD3+ cells than did the MNL populations from 3- or 4-d-old calves (P < 0.0002; Figure 5a). Percentages of γδ T cells in the MNL population from 1-d-old calves were higher than percentages in MNL populations from 2-, 3-, 4-, and 7-d-old calves and percentages in MNL populations from newborn calves (P < 0.0005; Figure 5b). Mononuclear leukocytes isolated from 1-d-old calves had higher percentages of B cells than did MNL from calves at birth and MNL from calves at 6- and 7-d of age (P < 0.0007; Figure 5c). Conversely, the percentage of MHC class II+ cells in MNL populations from 1-d-old calves was lower than that in MNL populations from 2-, 3-, and 4-d-old calves (P = 0.0001; Figure 5d).

A strong positive correlation between percentages of monocytes and MHC class II+ cells was detected (r = 0.81; P = 0.0001), and negative correlations between MHC class II+ antigen cells and CD3+ (r = -0.47; P = 0.0001), and CD4+ cells (r = -0.46; P = 0.0001) were detected.

Mononuclear populations from neonatal calves had significantly higher percentages of CD4+ and CD8+ T cells, γδ T cells, and monocytes, and lower percentages of B cells compared with MNL populations from adult cows (P ≤ 0.05; Table 2).

**In Vitro Secretion of IgM by MNL**

Cultures of MNL from neonatal calves were unresponsive to mitogenic stimulation in terms of secretion of IgM. These cultures secreted a mean 2.7 ± 0.5 ng/ml of IgM, which remained relatively unchanged over the 7-d period. Although secretion of IgM by

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Figure 3. Concentrations of 25-hydroxyvitamin D [25(OH)D] (a) and 1,25-dihydroxyvitamin D [1,25(OH)₂D] (b) in serum of neonatal calves treated with normal (◊) or skimmed (●) colostrum and milk for 7 d. Abscissa represents the age of the calves from which the serum was isolated. Means with different superscripts differ (P < 0.01).

Figure 4. Concentrations of IgG₁ in serum of neonatal calves treated with normal (◊) or skimmed (●) colostrum and milk for 7 d. Abscissa represents the age of the calves from which the serum was isolated.
unstimulated cell cultures from adult cows was similar to secretion by unstimulated cell cultures from calves, cells from adult cows that were stimulated with PWM secreted ~5 to 6 times more IgM than did MNL from calves that were stimulated with PWM (Figure 6a).

**In Vitro DNA Synthesis by MNL**

In the calf, MNL stimulated with PWM secreted more (P = 0.0004) IFN-γ than did parallel, unstimulated cultures. Interferon-γ secretion by unstimulated calf MNL remained low and unchanged (<55 pg/ml) during the 7-d period. Cells from 1- to 4-d-old calves that were stimulated with PWM secreted less IFN-γ than did cells from precolostral (d 0) and 7-d-old calves (P = 0.003; Figure 6b).

Although IFN-γ secretion by unstimulated cells from adult cows was similar to secretion by unstimulated cells from neonatal calves, cells from adult cows secreted ~100-fold more IFN-γ when stimulated with PWM (8688 ± 1616 vs. 73 ± 4 pg/ml produced by MNL stimulated with PWM in the calf).

**In Vitro Secretion of IFN-γ by MNL**

In the calf, MNL stimulated with PWM or concanavalin A incorporated significantly greater amounts of [3H]-thymidine than did parallel, unstimulated cultures (P = 0.0001). In calves, synthesis...
of DNA in cell cultures stimulated with concanavalin A was greater than that in parallel cell cultures stimulated with PWM (P = 0.002; Figure 7b). Synthesis of DNA by unstimulated and mitogen-stimulated cultures of cells from 3- and 4-d-old calves was lower than synthesis by cells from calves at birth (d 0) (P = 0.0001; Figure 7a). The magnitude of DNA synthesis induced by mitogen was similar for cultures that employed cells from calves and adults (Figure 7b).

**DISCUSSION**

In this study, we have shown that it is possible to abrogate the normal and progressive elevation in the concentration of RA and other retinoids in serum that is observed in calves during the 1st wk postpartum by depriving newborn calves of the natural fat fraction of colostrum and milk. The treatment did not affect concentrations of IgG1 in serum or the composition and functional capacities of peripheral blood MNL from these calves. However, this treatment prevented the rise in fat-soluble vitamins and their metabolites in serum as was observed in control calves that were fed intact colostrum and milk. Although calves fed the diet based on coconut oil had lower concentrations of the cis isomers of RA in serum than did calves fed the control diet, concentrations of all-trans-RA in serum were comparable. Further studies are needed to elucidate these effects. The treatment did not affect concentrations of 25(OH)D in serum but caused lower concentrations of 1,25(OH)2D. The lack of effect of the treatment on concentrations of 25(OH)D in serum could have been due to the diet, which was not a significant source of this vitamin. The elevation in 1,25(OH)2D in serum as observed in 1- and 2-d-old calves is in agreement with earlier reports, which indicate that low concentrations of calcium in plasma at birth activate the 1-α-hydroxylase enzyme and cause the generation of 1,25(OH)2D from 25(OH)D (12, 16).

Treatment of neonatal calves with this diet based on coconut oil resulted in marked alterations in fat-soluble vitamins and their metabolites in serum without affecting either passive immunity or immune function. All calves were clinically normal during the experimental period. Thus, this treatment method could be an appropriate model system to characterize further the events in vitamin A metabolism in the neonatal calf when the other fat-soluble vitamins meet dietary requirements. This approach could also be used to study the role of specific fat-soluble vitamins in neonatal calf physiology.

Major changes in the composition and function of peripheral blood MNL were found for all calves during the 7-d period. These changes have not been previously reported. Conceivably, newborn calves had reserves of fat-soluble vitamins in the liver or adipose tissue that met their requirements during the short-term depletion of these vitamins as imposed by the treatment. Measurement of these vitamins in serum would not indicate the presence of these reserves. It is possible that cells of the immune system had an adequate supply of these vitamins, which could explain why the treatment did not affect the immune parameters monitored. Long-term treatment of preg-

<table>
<thead>
<tr>
<th>Leukocyte antigen</th>
<th>Antibody</th>
<th>Neonatal calves1 (Mean ± SEM)</th>
<th>Adult cows2 (Mean ± SEM)</th>
<th>P3</th>
</tr>
</thead>
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<tr>
<td>CD3+ T Cell</td>
<td>MM1A</td>
<td>47.0 ± 5.0</td>
<td>36.5 ± 2.9</td>
<td>NS4</td>
</tr>
<tr>
<td>CD4+ T Cell</td>
<td>GC50A1</td>
<td>20.0 ± 2.4</td>
<td>13.3 ± 0.9</td>
<td>0.05</td>
</tr>
<tr>
<td>CD8α</td>
<td>BAQ111A</td>
<td>28.0 ± 4.0</td>
<td>19.1 ± 1.5</td>
<td>0.03</td>
</tr>
<tr>
<td>γδ T Cell</td>
<td>CACT61A</td>
<td>23.0 ± 4.0</td>
<td>9.4 ± 1.0</td>
<td>0.0014</td>
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<tr>
<td>B Cell</td>
<td>BAQ155A</td>
<td>16.7 ± 2.4</td>
<td>45.0 ± 3.0</td>
<td>0.0001</td>
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<tr>
<td>Monocyte</td>
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<td>13.0 ± 1.6</td>
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<tr>
<td>MHC Class II</td>
<td>TH14B</td>
<td>46.0 ± 7.0</td>
<td>54.0 ± 4.0</td>
<td>NS</td>
</tr>
<tr>
<td>Interleukin-2 receptor</td>
<td>CACT108A</td>
<td>47.7 ± 7.0</td>
<td>63.0 ± 5.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

1 Mean percentage of cells from 12 calves, each sampled for 7 consecutive d after birth.
2 Mean percentage of cells from two adult cows, each sampled on 8 separate d.
3 Probability that calves differed from adults.
4 P > 0.05.
5 Major histocompatibility complex.
nant cows and their newborn calves with a diet that was free of fat-soluble vitamins would deplete any reserves the calves might have, allowing the effects on the immune system to be discovered.

Results from the phenotypic analysis of MNL populations from calves in this study support earlier reports of higher percentages of circulating γδ T cells (14) and lower percentages of B cells (24) in neonatal ruminants than in adult cows. Although mitogen-induced lymphoproliferative responses of calf MNL were similar to the responses of MNL from adults, functional capabilities (i.e., IFN-γ and IgM secretion) were much lower. Observations in humans and mice have indicated that neonates have a greater proportion of naïve T cells, T cells capable of suppressing Ig production by B cells (10), and also antigen-presenting cells with ineffective costimulatory function (35) and lower cytokine-producing capabilities than do adults (36), which might explain these results. In contrast to the strong positive correlation observed between the percentage of CD3+ and γδ T cells and the ability of the cultures of MNL from adult cows to secrete IFN-γ (25), MNL from 1-d-old calves had the highest percentages of CD3+ and γδ T cells but had a diminished capacity to produce IFN-γ. Qualitative differences between neonatal and adult lymphocytes mentioned previously could account for these differences.

A dramatic daily effect was observed in the ability of the calf MNL to undergo lymphocyte proliferation, in the ability of calf MNL to secrete IFN-γ in culture, and in the percentages of cells expressing specific antigens. There was a decline in the percentage of MHC class II+ cells in the MNL within the first few days after birth. In addition, there was a strong positive correlation between MHC class II+ cells and the percentages of monocytes in these MNL populations and a strong negative correlation between MHC class II+ cells and CD3+ and CD4+ lymphocyte subsets. Stimulation of bovine MNL with PWM preferentially enhanced proliferation of CD4+ T lymphocytes (11, 21). Thus, in this system, the CD4+ T cells were likely the major secretors of IFN-γ. An ineffective accessory cell function, i.e., lesser numbers of MHC class II+ antigen-presenting cells or lower ratios of antigen-presenting cells to T cells, might have contributed to the reduced proliferation and secretion of IFN-γ by MNL from these calves.

Colostrum contains higher concentrations of immunosuppressive factors, such as cortisol, prostaglandins, and transforming growth factor-β, than does milk (13), which might have contributed to the suppression in lymphocyte proliferation and IFN-γ production within the first 4 d after birth. Concentrations of 1,25(OH)2D in serum were also elevated (∼0.1 ng/ml) in these calves at 1 to 2 d after birth. Supplementation of cultures of MNL from adult cattle with similar concentrations of this compound inhibited cell proliferation, decreased the numbers of MHC class II+ and interleukin-2 receptor+ cells (28), and suppressed IFN-γ secretion (1). Calves also have elevated plasma corticosteroid concentrations at birth (≥8 μg/dl), which decrease to ≤4 μg/dl within 24 h (19). Corticosteroids have profound immunosuppressive effects, presumably because they inhibit the activity of the transcription factor nuclear factor κ B, which is needed for the expression of numerous molecules of the immune system (2). Dexamethasone suppresses IFN-γ and IgM secretion by peripheral

Figure 7. Mean incorporation of [3H]thymidine by peripheral blood mononuclear leukocytes (MNL) from neonatal calves (a). Abscissa represents the age of the calves from which the MNL were isolated. Mean incorporation of [3H]thymidine by peripheral blood MNL from neonatal calves and nongravid, nonlactating adult cows that were unstimulated (hatched bar) or stimulated with 1 μg/ml of pokeweed mitogen (open bar) or with 1 μg/ml of concanavalin A (solid bar) (b). Means with different superscripts differ (P ≤ 0.01).
blood leukocytes that are stimulated with mitogens in adult dairy cows (25). Thus, these different factors could have contributed to the observed decrease in IFN-γ secretion, or MHC class II expression, or both, as well as to the diminished lymphoproliferative responses of the MLN isolated from 1- to 4-d-old calves. The synthetic corticosteroid, dexamethasone, has been shown to affect expression of adhesion molecules in lymphocytes from 15-mo-old dairy bulls (6). Expression of adhesion molecules on peripheral blood MNL could have been influenced by circulating glucocorticoids in 1-d-old calves, which might have led to the retention of T and B lymphocytes in circulation, resulting in higher percentages of these populations in 1-d-old calves.

CONCLUSIONS

The diet based on coconut oil for newborn calves may be considered a model system that could be further manipulated and used to characterize the events in the metabolism of fat-soluble vitamins in the neonate. Also, the treatment could be extended over longer periods to study the effects of deficiency of one or more of these vitamins on immune responsiveness in calves.

These results suggest that there is a window during the first few days postpartum when several factors contribute to an immunosuppressed state in the calf, making it highly susceptible to infectious disease. This situation is similar to the immunosuppression seen in the dam in the postpartum period; calf MLN regain their functional and proliferative abilities by the end of the 7-d period. Dramatic physiologic changes occur in the neonatal calf in the first few days after birth. A clearer understanding of these events would increase our knowledge of the health and productivity of cows.

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